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Early Detection of Abnormal Prion Protein in Genetic Human Prion Diseases Now Possible Using Real-Time QUIC Assay

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Abstract

Introduction: The definitive diagnosis of genetic prion diseases (gPrD) requires pathological confirmation. To date, diagnosis has relied upon the finding of the biomarkers 14-3-3 protein and total tau (t-tau) protein in the cerebrospinal fluid (CSF), but many researchers have reported that these markers are not sufficiently elevated in gPrD, especially in Gerstmann-Sträussler-Scheinker syndrome (GSS). We recently developed a new *in vitro* amplification technology, designated "real-time quaking-induced conversion (RT-QUIC)", to detect the abnormal form of prion protein in CSF from sporadic Creutzfeldt-Jakob disease (sCJD) patients. In the present study, we aimed to investigate the presence of biomarkers and evaluate RT-QUIC assay in patients with gPrD, as the utility of RT-QUIC as a diagnostic tool in gPrD has yet to be determined.

Method/Principal Findings: 56 CSF samples were obtained from gPrD patients, including 20 cases of GSS with P102L mutation, 12 cases of fatal familial insomnia (FFI; D178N), and 24 cases of genetic CJD (gCJD), comprising 22 cases with E200K mutation and 2 with V203I mutation. We subjected all CSF samples to RT-QUIC assay, analyzed 14-3-3 protein by Western blotting, and measured t-tau protein using an ELISA kit. The detection sensitivities of RT-QUIC were as follows: GSS (78%), FFI (100%), gCJD E200K (87%), and gCJD V203I (100%). On the other hand the detection sensitivities of biomarkers were considerably lower: GSS (11%), FFI (0%), gCJD E200K (73%), and gCJD V203I (67%). Thus, RT-QUIC had a much higher detection sensitivity compared with testing for biomarkers, especially in patients with GSS and FFI.

Conclusion/Significance: RT-QUIC assay is more sensitive than testing for biomarkers in gPrD patients. RT-QUIC method would thus be useful as a diagnostic tool when the patient or the patient's family does not agree to genetic testing, or to confirm the diagnosis in the presence of a positive result for genetic testing.

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Introduction

Prion diseases (PrD) are fatal neurodegenerative disorders characterized by the accumulation of abnormal prion protein (PrP^{Sc}) in the CNS. The genetic form of human PrD (gPrD) is caused by mutations in the *prion protein gene* (*PRNP*), and is classified into genetic CJD (gCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Patients with GSS and FFI have symptoms such as dementia, dyskinesia and sleep disorders, but show no specific signal in diffusion-weighed MR imaging, and therefore the clinical discrimination of GSS and FFI from non-prion diseases such as spinocerebellar degeneration (SCA) [1] and chronic refractory sleep disorders, respectively, is problematic.

Table 1. Summary of CSF analysis of genetic prion disease cases.

	GSS	FFI	g CJD	
	P102L	D178N	E200K	V203I
Number	20	12	22	2
Age (average year)	55.5±4.45	55.8±13.7	62.7±9.43	73
Sex (m:f)	1:3	3:1	1:1	2:0
positive patients/total (%)				
[95% CI*]				
	4/20	1/12	19/22	1/2
t-tau protein	(20%)	(8.3%)	(86.3%)	(50%)
	[2.6–37.4%]	[0–25.4%]	[70.2–100%]	
	4/20	1/12	18/22	1/2
14-3-3 protein	(20%)	(8.3%)	(81.8%)	(50%)
	[2.6–37.4%]	[0–25.4%]	[67.4–96.2%]	
	18/20	10/12	18/22	2/2
RT-QUIC	(90%)	(83.3%)	(81.8%)	(100%)
	[76.5–100%]	[70.2–100%]	[67.4–96.2%]	

*The 95% confidence interval [CI] was calculated using the adjusted Wald test, and was expressed only in groups of more than seven cases.

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We recently developed a new *in vitro* amplification technology, designated "real-time quaking-induced conversion (RT-QUIC)", for the detection of PrP^{Sc} in CSF of sCJD [2]. The aim of the present study was to determine whether RT-QUIC could also be of value in patients with genetic prion disease, as well as in sCJD.

Materials and Methods

Patients

We retrospectively analyzed 56 CSF samples obtained from gPrD patients in Japan, South Korea and Germany, including 22 cases of E200K gCJD, 20 cases of P102L GSS, 12 cases of FFI and 2 cases of V203I gCJD (Table1). PrP-genotyping was done using genomic DNA extracted from peripheral blood leukocytes, as described previously [3]. Informed consent was obtained from patients' families and/or patients. The study protocol was approved by the Ethics Committee of Nagasaki University Hospital (ID: 10042823) and registered with the University Hospital Medical Information Network (ID: UMIN000003301).

Real-time QUIC and analysis of 14-3-3 and t-t-tau protein in CSF samples

We analyzed all CSF samples by RT-QUIC method as previously described [2]. 14-3-3 proteins in CSF were analyzed by Western blotting and total-tau protein was measured using an ELISA kit (INNOTEST®) as previously described [3].

Expression and purification of recombinant human PrP

Recombinant PrP, equivalent to residues 23–231 of the human PrP sequence, (codon 129 M) was expressed, refolded into a soluble form (rHuPrP-sen), and purified essentially as described previously [2]. The concentration of rHuPrP-sen was determined by measuring the absorbance at 280 nm. The purity of the final protein preparations was ≥99%, as estimated by SDS-PAGE, immunoblotting, and liquid chromatography-mass spectrometry. Circular dichroism analysis showed the conformation of rHuPrP-sen was α-helix-rich (data not shown). After purification, aliquots of the proteins were stored at −80°C in 10 mM phosphate buffer, pH 6.8.

Real-time QUIC

We prepared reactions in a black 96-well optical bottom plate (Nunc, Rochester, NY, USA) to a final volume of 100 µl. To avoid contamination, we prepared non-infectious materials inside a biological safety cabinet in a prion-free laboratory and used aerosol-resistant tips. The final concentrations of reaction buffer components were 500 mM NaCl, 50 mM PIPES pH 7.0, 1 mM EDTA and 10 µM Thioflavin T. The rHuPrP-sen concentration was 50 µg/ml, and only freshly-thawed rHuPrP-sen was used. CSF (5 µl per well) was used to seed the RT-QUIC reactions. The 96-well plate was covered with sealing tape (Nunc 236366) and incubated at 37°C in a plate reader (Infinite M200 or F200 fluorescence plate reader; TECAN) with intermittent shaking, consisting of 30 s circular shaking at the highest speed and no shaking for 30 s, with a 2 min pause to measure the fluorescence. The kinetics of fibril formation was monitored by reading the fluorescence intensity every 10 min using 440 nm excitation and 485 nm emission and monochromators (Infinite M200) or filters (Infinite F200).

Results (Figure 1, Table 1, 2 and 3)

First we analyzed 22 CSF samples from E200K gCJD patients. The positivities of t-tau protein, 14-3-3 protein and RT-QUIC method were all in the range of 80–85% (Figure 1, Table 1, 2 and 3). Overall, PrP^{Sc} was detected in 18 of the cases by RT-QUIC, all of which were also positive for both t-tau and 14-3-3 proteins. In the GSS and FFI cases, RT-QUIC was positive in 90% of GSS and 83.3% of FFI (Figure 1 and Table1 and 2). Among the GSS cases, however, 80% showed negative for both t-tau and 14-3-3 proteins, and all but one of the FFI samples were negative for the biomarkers (Figure 1, Tables 1 and 2). Although we were able to analyze only 2 cases of gCJD V203I, both were positive by RT-QUIC and only one was positive for the biomarkers. All gPrD patients were methionine homozygotes at codon 129 of *PRNP*.

We compared the kinetics of recombinant Human PrP (rHuPrP) fibril formation in CSF of sCJD patients with those of gPrD patients, and found no significant difference (Figure 1).

Discussion

The RT-QUIC in vitro PrP^{Sc} amplification assay for diagnosis of prion disease has shown 84% sensitivity and 100% specificity in CSF samples from sporadic CJD patients.

To determine the value of RT-QUIC in genetic prion disease diagnosis, we analyzed a total of 56 CSF samples from patients with various genetic forms of human prion disease (Table 1). Our study demonstrated that RT-QUIC was highly positive in all four of the gPrD types we analyzed. Notably, most of the GSS and FFI patients were negative for both 14-3-3 and t-tau, as found in previous studies [4], whereas RT-QUIC showed 90% positivity in GSS.

RT-QUIC method was capable of detecting an extremely low volume of PrP^{Sc}, and we were able to detect as little as ≥1 fg of PrP^{Sc} in diluted brain homogenate from sCJD patients. While we were not able to detect PrP^{Sc} in all sCJD CSF samples, the reasons for this are unclear and we assume that the amounts of PrP^{Sc} are very much lower in CSF samples of negative gPrD patients.

Because the majority of GSS patients remain alive with only relatively mild symptoms one year after the onset [5], many

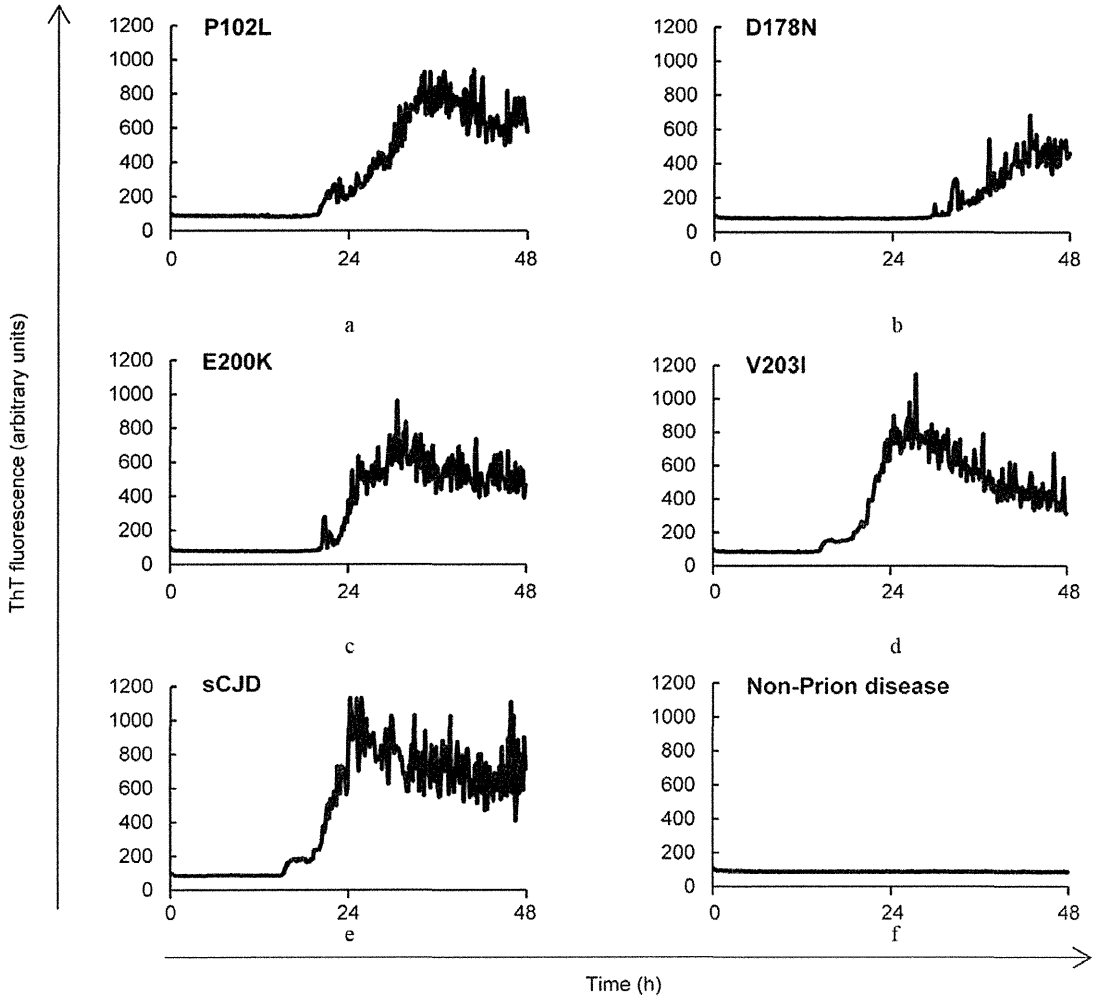

Figure 1. The kinetics of rHuPrP fibril formation with seeds from CSF of GSS, FFI, or gCJD. (**a**) a GSS P102L patient (**b**) a FFI D178N patient (**c**) a gCJD E200K patient (**d**) a gCJD V203I patient (**e**) a sCJD (MM1) patient and (**f**) a control subject.
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Table 2. Further analysis of CSF samples of P102L GSS patients.

	duration between the symptom onset and lumbar puncture	
	1–12 months	13–77 months
Number	8 samples	12 samples
Age (average year)	56.8±2.14	54.9±5.19
Sex (m:f)	3:5	2:10
positive patients/total (%)		
t-tau protein	3/8	1/12
	37.5%	8.3%
14-3-3 protein	3/8	1/12
	37.5%	8.3%
RT-QUIC	8/8	10/12
	100%	83.3%

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Table 3. Further analysis of CSF samples of gCJD E200K patients.

	duration between the symptom onset and lumbar puncture	
	1–3 months	4–48 months
Number	10 samples	12 samples
Age (average year)	61.4±8.96	63.8±9.67
Sex (m:f)	7:3	1:2
positive patients/total patients(%)		
t-tau protein	9/10	10/12
	90.0%	83.3%
14-3-3 protein	8/10	10/12
	80.0%	83.3%
RT-QUIC	7/10	11/12
	70.0%	91.6%

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consult a clinician only later in the disease progression. On other hand, the progression of CJD is much more rapid, with most patients exhibiting akinetic mutism within 3 months. For this reason, we define the "early stage" in GSS (P102L) as 1–12 months, and in E200K gCJD as 0–3 months.

Moreover GSS and FFI show considerable phenotypic variability [6,7], and it is very important to distinguish them from non-prion diseases at an early stage. Until now, this has not been possible. Using the RT-QUIC assay, however, we were able to confirm positivity in 100% of GSS patients at an early stage, prior to disease progression (Table 2). Thus, RT-QUIC has application in the laboratory detection of gPrD as well as sCJD, and is likely to be of particular advantage in the differential diagnosis of FFI and GSS, in which biomarkers are usually negative (Tables 2 and 3).

Interestingly, one patient with E200K gCJD was negative by RT-QUIC when sampled at 2 months after the symptom onset, but became positive when a second sample was obtain two months later. Thus, it is important that even if the CSF analysis by RT-QUIC is negative at an early stage, it should be re-examined at a later time point. Additionally, the use of RT-QUIC along with testing for the biomarkers should prove valuable for monitoring clinical trials of therapeutic agents use in gPrD patients.

We believe that RT-QUIC analysis of CSF will become invaluable in the differential diagnosis of suspected prion diseases, since not all patients with the genetic mutation go on to develop prion diseases. Alpha

In conclusion, RT-QUIC enables the early diagnosis of GSS and FFI in many patients for whom a differential diagnosis is otherwise not currently possible.

Author Contributions

Conceived and designed the experiments: K. Satoh K. Sano RA NN. Performed the experiments: K. Sano K. Satoh RN. Analyzed the data: K. Satoh. Contributed reagents/materials/analysis tools: HT YI MY NS H. Murai H. Mizusawa MS IZ YK. Wrote the paper: K. Sano K. Satoh RN NN.

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RESEARCH PAPER

Insight into the frequent occurrence of dura mater graft-associated Creutzfeldt-Jakob disease in Japan

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ABSTRACT

Objective More than 60% of patients worldwide with Creutzfeldt-Jakob disease (CJD) associated with dura mater graft (dCJD) have been diagnosed in Japan. The remarkable frequency of dura mater grafts in Japan may possibly contribute to the elevated incidence of dCJD, but reasons for the disproportionate use of this procedure in Japan remain unclear. We investigated differences between dCJD patients in Japan and those elsewhere to help explain the more frequent use of cadaveric dura mater and the high incidence of dCJD in Japan.

Methods We obtained data on dCJD patients in Japan from the Japanese national CJD surveillance programme and on dCJD patients in other countries from the extant literature. We compared the demographic, clinical and pathological features of dCJD patients in Japan with those from other countries.

Results Data were obtained for 142 dCJD patients in Japan and 53 dCJD patients elsewhere. The medical conditions preceding dura mater graft transplantation were significantly different between Japan and other countries ($p<0.001$); in Japan, there were more cases of cerebrovascular disease and hemifacial spasm or trigeminal neuralgia. Patients with dCJD in Japan received dura mater graft more often for non-life-threatening conditions, such as meningioma, hemifacial spasm and trigeminal neuralgia, than in other countries.

Conclusions Differences in the medical conditions precipitating dura mater graft may contribute to the frequent use of cadaveric dura mater and the higher incidence of dCJD in Japan.

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To date, 469 cases of iatrogenic Creutzfeldt-Jakob disease (CJD) have been reported worldwide.[1] The medical procedures resulting in transmission of CJD include cadaveric dura mater graft transplantation, neurosurgery, EEG needle use, corneal transplantation, human cadaveric pituitary hormone administration and blood transfusion.[1 2] Nearly half of the iatrogenic CJD cases are associated with dura mater grafts.[1 2] More than 60% (142 cases) of patients worldwide with dura mater graft-associated CJD (dCJD) are from Japan.[1 2]

Although the exact number of patients receiving a dura mater graft is unknown, it has been estimated that cadaveric dura mater was used in approximately 20 000 Japanese patients annually during the 1980s.[3] The extremely frequent use of dura mater grafts in Japan was much higher than in any other country,[4] although the reasons for this remain unclear. In this study, we investigated differences between patients with dCJD in Japan and elsewhere to help explain the common use of cadaveric dura mater and the high incidence of dCJD in Japan.

METHODS

Patients with dCJD in Japan

We obtained data on patients with dCJD in Japan from previously reported nationwide surveillance studies, including a nationwide questionnaire survey carried out by the Japan CJD Surveillance Group to identify CJD patients.[3 5–11] More detailed information was obtained by a member of the surveillance group who visited the referral hospital or conducted a post-mortem review of the case records of patients with a history of dura mater graft transplantation.[5] Between 1996 and 1999, additional dCJD patients were identified.[5] In April 1999, prospective surveillance of human prion disease by the Creutzfeldt-Jakob Disease Surveillance Committee was initiated,[6–10] where each case of suspected prion disease was investigated by members of the committee in cooperation with CJD specialists in each prefecture. The study protocol was approved by the medical ethics committee of Kanazawa University and Tokyo Medical and Dental University.

Patients with dCJD in countries other than Japan

Literature searches of PubMed and Embase were conducted to identify patients with dCJD in countries other than Japan. Search terms included 'Creutzfeldt-Jakob disease' and 'dura' or 'dural'. We selected articles that provided clinical information about patients in the English language; conference abstracts were excluded.

Data analyses

We analysed sex, age and calendar year at dura mater graft transplantation, medical conditions leading to the use of dura mater grafts, age and calendar year at CJD onset, incubation period between dura mater graft transplantation and CJD onset, polymorphism at codon 129 of the prion protein gene (*PrP*), the source of the dura mater, and pathological data. Patients with dCJD were classified into two different types, non-plaque and plaque.[6] Non-plaque type dCJD shows the classic clinicopathological features of sporadic CJD

(sCJD), while plaque-type dCJD is characterised by a relatively slow progression of neurological manifestations and the presence of amyloid plaques.[6]

Statistical analysis

Differences between Japan and other countries in sex distribution, use of Lyodura (B Braun, Melsungen, Germany; the dura mater graft material used in most patients with dCJD), medical conditions requiring dura mater graft transplantation, polymorphism at codon 129 of *PrP* and neuropathological type were analysed with Fisher's exact probability test. Differences between Japan and other countries in age at dura mater graft transplantation, age at CJD onset and incubation period between dura mater graft transplantation and CJD onset were analysed with the Mann–Whitney U test. Differences in incubation period as regards *PrP* codon 129 polymorphism were analysed with one-way analysis of variance. Statistical significance was defined as $p<0.05$. Statistical analyses were performed using SPSS V.19 (IBM, Armonk, New York, USA).

RESULTS

As of June 2012, we had identified 142 patients with dCJD in Japan, and obtained from published articles information on 53 other patients diagnosed with dCJD elsewhere (see online supplementary e-references 1–31). The numbers of patients with dCJD in each country analysed in this study are shown in online supplementary table e-1. Except for Japanese patients, most cases of dCJD were in western countries.

The clinical, genetic and pathological features were compared between patients in Japan and elsewhere (table 1). The proportion of patients undergoing dura mater graft transplantation because of medical conditions was significantly different between Japan and other countries ($p<0.001$). The most frequent precipitating medical condition was tumours in both Japan and other countries. Vascular diseases and hemifacial spasm or trigeminal neuralgia were more frequent as causes in Japan (vascular diseases: 28.9% and hemifacial spasm or trigeminal neuralgia: 18.3%) compared to other countries (6.1% and 2.0%, respectively). The mean age at receiving a dura mater graft in Japan was significantly higher than in other countries ($p<0.001$), as was the mean age at CJD onset ($p<0.001$). The incubation period between dura mater graft transplantation and CJD onset was not significantly different between Japan and other countries. We identified the source of cadaveric dura mater in 130 (91.5%) patients in Japan and 38 (71.7%) patients in other countries, and found that Lyodura is used significantly more often in Japan than in other countries ($p<0.05$). Besides Lyodura, Zenoderm (porcine) was used for a patient in the UK,[12] Tutoplast for a patient in the USA[13] and non-commercial dura mater for a patient in Italy.[14] We obtained the results of the polymorphic *PrP* codon 129 analyses for 59 (41.5%) patients in Japan and 35 (66.0%) patients in other countries. The proportion of each genotype was significantly different between Japan and other countries ($p<0.01$), showing a higher frequency of the methionine homozygote in Japan. The distribution of pathology type (non-plaque or plaque) was not significantly different between Japan and other countries.

In order to clarify why age at dura mater graft transplantation in Japan was higher than in other countries, we examined each medical condition leading to the use of dura mater graft by age (table 2). Patients who underwent neurosurgery for tumours in Japan were approximately 12 years older than those in other countries. Patients who had surgery for hemifacial spasm or trigeminal neuralgia were approximately 50 years of age in both Japan and other countries.

We analysed the types of tumours in these patients (table 3). In Japan, meningioma was the most frequent tumour requiring a dura mater graft (48.4%), followed by acoustic neurinoma (27.4%). In other countries, glioma was the most frequent tumour (34.6%), followed by meningioma (30.8%) and acoustic neurinoma (11.5%).

Table 1 Comparison of clinical, genetic and pathological features of patients with dura mater graft-associated CJD (dCJD) between Japan and other countries

	Japan	Other countries	p Value
Patients (n)	142	53	
Sex, female (%)	58.5	43.5	NS
Diseases preceding dura mater graft, % (n)			
Tumour	43.7 (62/142)	53.1 (26/49)	p<0.001
Vascular diseases	28.9 (41/142)	6.1 (3/49)	
Trauma	4.2 (6/142)	12.2 (6/49)	
Hemifacial spasm or trigeminal neuralgia	18.3 (26/142)	2.0 (1/49)	
Others	4.9 (7/142)	26.5 (13/49)	
Age at receiving dura mater graft* (range in years)	43.1±15.1 (1–70)	31.6±15.0 (5–61)	p<0.001
Age at CJD onset* (range in years)	55.7±14.9 (15–80)	42.9±15.6 (16–73)	p<0.001
Incubation period* (range in years)	12.1±5.8 (1–30)	11.3±6.0 (1–23)	NS
Proportion using Lyodura, % (n)	100 (130/130)	92.1 (35/38)	p<0.05
Codon 129 polymorphism, % (n)			
Met/Met	96.6 (57/59)	74.3 (26/35)	p<0.01
Met/Val	3.4 (2/59)	8.6 (3/35)	
Val/Val	0 (0/59)	17.1 (6/35)	
Pathology type, % (n)			
Non-plaque	54.8 (17/31)	78.9 (15/19)	NS
Plaque	45.2 (14/31)	21.1 (4/19)	

*Values are mean±SD.
CJD, Creutzfeldt-Jakob disease; Met, methionine; NS, not significant; Val, valine.

Table 2 Age of patients in Japan and other countries with each medical condition at dura mater graft

	Japan (years), mean±SD	Other countries (years), mean±SD
Tumour	43.8±13.8 (n=62)	30.8±16.7 (n=26)
Vascular diseases	40.2±17.7 (n=41)	35.7±6.1 (n=3)
Trauma	24.5±17.2 (n=6)	27.8±15.1 (n=6)
Hemifacial spasm or trigeminal neuralgia	50.2±9.4 (n=26)	50 (n=1)
Others	44.3±10.4 (n=6)	29.1±14.0 (n=13)

Table 4 Incubation period from dura mater graft to the onset of Creutzfeldt-Jakob disease in each prion protein codon 129 genotype

	Japan (years), mean±SD	Other countries (years), mean±SD	Total (years), mean±SD
Met/Met	16.4±4.9 (n=56)	12.7±6.2 (n=26)	15.2±5.6 (n=82)
Met/Val	13.0±4.2 (n=2)	13.0±7.0 (n=3)	13.0±5.4 (n=5)
Val/Val	–	14.0±4.6 (n=6)	14.0±4.6 (n=6)

Met, methionine; Val, valine.

We investigated the incubation period between dura mater graft transplantation and CJD onset in each *PrP* codon 129 genotype (table 4). The incubation period was not significantly different among these genotypes in the entire sample in Japan or in other countries.

Patients in Japan received dura mater grafts from 1975 to 1993, and in other countries from 1969 to 1992 (figure 1A). The years from 1981 to 1987 were the highest risk period for dCJD following dura mater grafting in patients both in Japan and elsewhere (figure 1A). The year of CJD onset ranged from 1985 to 2009 in Japan and from 1978 to 2010 in other countries, while many patients developed dCJD from 1985 to 2006 in both Japan and elsewhere (figure 1B).

DISCUSSION

Our results show that patients with dCJD in Japan had several notable features compared to those in other (primarily western) countries: (i) cerebrovascular diseases and hemifacial spasm or trigeminal neuralgia were frequent reasons for dura mater graft transplantation; (ii) patients receiving a dura mater graft were older; (iii) Lyodura was more frequently used as grafted dura mater; and (iv) more patients were homozygous for methionine at *PrP* codon 129.

The medical conditions precipitating dura mater graft were significantly different between Japan and other countries (table 1). The death rate from stroke was higher in Japan than in western countries, while that from coronary heart disease was lower.[15] In addition, the incidence of stroke in Japan was the highest among developed countries in the 1960s.[15] Although death rates from stroke in Japan decreased from 1960 to 1990, the incidence of stroke was still higher than that of coronary heart disease in Japan in 1980.[15 16] Thus, the high incidence of stroke might have contributed to the high proportion of vascular diseases among the medical conditions leading to dura mater grafting in Japan. Hemifacial spasm or trigeminal neuralgia was also more frequent in Japan (18.3%) than in other countries (2.0%; table 1). In this diagnostic sub-group, microvascular decompression surgery was performed.[17–19] According to a

Table 3 Detailed diagnoses of patients who underwent neurosurgery for a tumour in Japan and in other countries

	Japan, n (%)	Other countries, n (%)
Meningioma	30 (48.4)	8 (30.8)
Acoustic neurinoma	17 (27.4)	3 (11.5)
Glioma	2 (3.2)	9 (34.6)
Ependymoma	2 (3.2)	0 (0)
Hemangioblastoma	2 (3.2)	2 (7.7)
Spinal cord tumour	2 (3.2)	1 (3.8)
Others	7 (11.3)	3 (11.5)

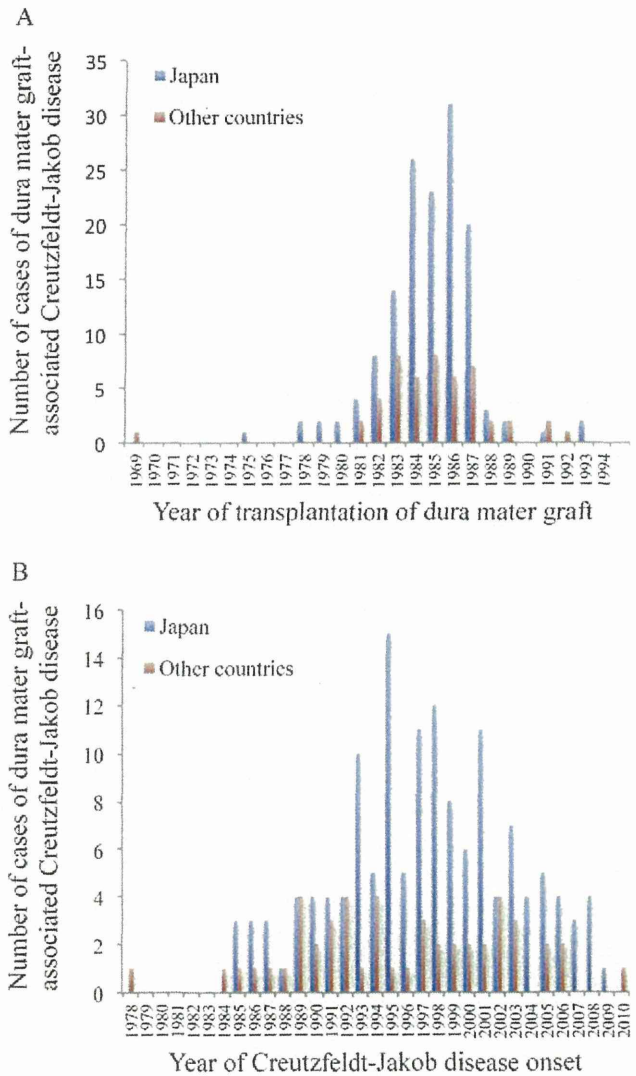

Figure 1 Number of patients with dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) by year of dura mater graft procedure (A) and by year of dCJD onset (B). (A) Patients in Japan underwent dura mater graft transplantation from 1975 to 1993, while patients in other countries underwent surgery from 1969 to 1992. Most patients in both Japan and other countries received dura mater grafts between 1981 and 1987. (B) Patients in Japan developed dCJD from 1985 to 2009, while those in other countries developed dCJD from 1978 to 2010. The majority of all patients developed dCJD between 1985 and 2008, while the number of diagnoses of new-onset dCJD continues to decrease in Japan and elsewhere.

Japanese review of this procedure, use of Lyodura was recommended to patch the opened dura at the posterior fossa,[20] which likely contributed to the frequent use of Lyodura by Japanese neurosurgeons.

Patients receiving a dura mater graft in Japan were significantly older than those elsewhere (table 1). Tumours were the most frequent medical condition in both Japan and other countries (table 1), but age at surgery for tumours was approximately 12 years higher in Japan than in other countries (table 2). Previous epidemiological surveys indicated that the incidence of glioma in Japan was almost half of that in western countries,[21 22] and that glioma rates were higher in white than in black, Latin and Asian subjects.[23] In addition, although glioma was more frequent than meningioma in western countries, the reverse was true in Japan.[21] The incidence of meningioma increased until 79 years of age, although a similar increase in the incidence of other types of tumour including glioma was not evident.[21] Thus, the high incidence of meningioma in Japan may explain the older age at dura mater graft transplantation. Furthermore, the frequent use of dura mater grafting for hemifacial spasm or trigeminal neuralgia in Japan may also explain the higher age at dura mater graft transplantation, since age at surgery for these conditions was the highest among all surgeries for dura mater graft transplantation (table 2).

Most patients (165/168 patients in total; 98.2%) in whom the source of cadaveric dura mater could be identified, received Lyodura (table 1). It was estimated that about 20 000 grafts were used each year in Japan during the 1980s,[3] and only Lyodura was available for dura mater grafting in Japan from July 1973 to August 1985. The cumulative incidence rate of dCJD in Japan was 0.09% in neurosurgical patients receiving a dura mater graft between 1981 and 1987, with an incidence rate of 0.15% during the peak year of 1986.[3] In contrast, less than 2500 patients received Lyodura in Australia and the cumulative risk of dCJD there was 0.20–0.23%.[24] In addition, in the USA, it was estimated that less than 400 patients per year received Lyodura,[4] and the cumulative incidence rate of dCJD there was 0.11% between 1981 and 1987. These results indicate that it is unlikely that the Lyodura used in Japan was more contaminated with abnormal prion protein than that used in other countries.

Regarding the polymorphism at codon 129 of *PrP* in dCJD, the distribution in Japan and other countries is similar to that in sCJD, as a recent study reported.[1] In the general Japanese population, the frequency of methionine homozygotes is higher and that of other genotypes lower (methionine homozygotes: 0.92; methionine/valine heterozygotes: 0.08; valine homozygotes: 0) compared to European populations (methionine homozygotes: 0.37–0.49; methionine/valine heterozygotes: 0.42–0.49; valine homozygotes: 0.08–0.15).[25–31] Although homozygosity at *PrP* codon 129 predisposes to sporadic and iatrogenic CJD,[25 32 33] the cumulative incidence rate of dCJD in Japan (0.09%) was no higher than that in Australia (0.20–0.23%)[24] or the USA (0.11%), as mentioned previously. Thus, the frequent occurrence of methionine homozygotes in the general Japanese population might not explain many cases of dCJD in Japan.

The genotype of the polymorphism at *PrP* codon 129 influences the clinicopathological phenotypes of dCJD, and all patients with plaque-type dCJD ever reported have been methionine homozygotes.[6–8] An animal study showed that plaque-type dCJD pathology could be caused by cross-sequence transmission of sCJD-VV2 prions to individuals from patients with the methionine homozygote.[34] Furthermore, another animal study showed that sCJD-MV2 prions could also induce plaque-type dCJD pathology (T Kitamoto, unpublished data, April 2013). The proportion of sCJD-VV2 and sCJD-MV2 patients among all cases of sCJD in Germany, where Lyodura was produced, is 24.3%.[35] The frequency of plaque-type dCJD in Japan (45.2%) as reported in the current study seems inordinately high. One possible reason is that the number of patients in whom pathological type could be confirmed was relatively small in Japan (21.8%) and atypical cases (plaque type) underwent autopsy more frequently than typical cases (non-plaque type). In our previous reports which included clinically diagnosed cases, about one-third of patients with dCJD may have the plaque type,[6 8] which is similar to the proportion of sCJD-VV2 and sCJD-MV2 in Germany.

Although age at dura mater graft transplantation and the distribution of the *PrP* codon 129 polymorphism were different between patients in Japan and in other countries, the incubation period between dura mater graft transplantation and CJD onset was similar among all patients (table 1). Interestingly, although the incubation period was not significantly different among the three codon 129 genotypes in dCJD patients in the current study (table 4), homozygosity has been reported to be associated with a shorter incubation period in human growth hormone-associated CJD.[36]

In both Japan and other countries, dura mater graft transplantation from 1981 to 1987 was associated with the highest risk of dCJD (figure 1A). Until May 1987, cadaveric dura mater was treated with 10% H_2O_2 for 24 h and irradiated with gamma rays (25 kGy). However, after May 1987, when the first case of dCJD was reported, the manufacturer included treatment with 1N NaOH for 1 h to reduce the risk of CJD transmission.[37 38] Nevertheless, a small number of patients with dCJD received a dura mater graft in or after 1988 in both Japan and other countries (figure 1A). Most patients developed CJD between 1985 and 2006, while the number of new-onset dCJD cases has decreased since approximately 2002 in Japan and elsewhere (figure 1B).

The high incidence of stroke and the frequent use of a dura mater graft for hemifacial spasm or trigeminal neuralgia may both play a role in the markedly higher use of dura mater graft procedures in Japan compared to elsewhere. In addition, patients in Japan may have received Lyodura more frequently as it was covered by national health insurance.[39] Furthermore, results from the present study show that patients with dCJD in Japan received a dura mater graft more often for non-life-threatening conditions than patients in other countries. Although surgery for a tumour was the most frequent reason for a dura mater graft in Japan and elsewhere (table 1), the proportions of meningioma and acoustic neurinoma were higher and that of glioma lower in Japan compared to other countries (table 3). Meningioma and acoustic neurinoma normally have a good prognosis, while glioma is sometimes fatal. Furthermore, in Japan many patients received a dura mater graft following microvascular decompression surgery for hemifacial spasm or trigeminal neuralgia (table 1), and most survived until they developed CJD in old age.

Several limitations should be considered when reviewing the results of this study. First, data on patients with dCJD in other countries was limited to information available in the extant literature. Second, the method of obtaining patient information differed in Japan from that in other countries. However, information from most journal articles was detailed enough for comparison of data on patients from Japan with data from other countries.

In conclusion, this study elucidated several important differences in the dura mater graft patient population in Japan

compared to other countries. The different medical conditions precipitating dura mater graft transplantation might have increased the use of Lyodura in Japanese patients, and patients in Japan may be more likely to have survived until CJD developed because they received a dura mater graft more often for non-life-threatening conditions than patients in other countries.

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Graft-related disease progression in dura mater graft-associated Creutzfeldt-Jakob disease: a cross-sectional study

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ABSTRACT

Objectives: Details of abnormal prion protein (PrP^{Sc}) propagation in the human central nervous system (CNS) are unclear. To assess the spread of PrP^{Sc} through the human CNS, we evaluated dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) cases focusing on sites of grafting and dCJD pathological subtypes.

Design: A cross-sectional study.

Setting: nationwide surveillance data of human prion diseases in Japan over the past 12 years were applied for the study.

Participants: Clinical data were obtained from 84 dCJD patients.

Outcome measures: The clinical courses in cases of dCJD were analysed according to the grafting sites (supratentorial and infratentorial groups) and the pathological subtypes (non-plaque and plaque types).

Results: Of the 84 cases of dCJD in this study, 36 (43%) were included in the supratentorial group and 39 (46%) were included in the infratentorial group. As initial manifestations, vertigo (p=0.007) and diplopia (p=0.041) were significantly more frequent in the infratentorial group than in the supratentorial group. During their clinical course, cerebellar signs appeared more frequently in the infratentorial group than in the supratentorial group (p=0.024). In the non-plaque type cases (n=53), the infratentorial group developed vertigo more frequently than the supratentorial group (p=0.017); moreover, cerebellar signs appeared more frequently in the infratentorial group (p=0.014). However, there was no significant difference between groups in the plaque type (n=18).

Conclusions: The high frequency of clinical manifestations related to brain stem and cerebellar dysfunction in the non-plaque type dCJD with infratentorial grafting suggests that PrP^{Sc} commonly shows direct propagation into the CNS from contaminated dura mater grafts.

ARTICLE SUMMARY

Article focus

- Evaluation of the relationship between initial manifestation and the site of dura mater graft in patients with dura mater graft-associated Creutzfeldt-Jakob disease (dCJD).
- We analysed the clinical data taking into account not only the grafting site but also the pathological subtypes of dCJD.

Key messages

- Infratentorial grafting cases in non-plaque type developed manifestations related to dysfunction of the brain stem and cerebellum more frequently than did supratentorial grafting cases.
- Cerebellar signs also appeared more frequently in the infratentorial group during their clinical course.
- Plaque-type cases showed no significant difference between the supratentorial and the infratentorial groups.

Strengths and limitations of this study

- This study suggests that the non-plaque-type abnormal prion protein (PrP^{Sc}) strain would propagate directly from the grafted dura mater to the adjacent brain and damage it earlier and more severely.
- It would be difficult to determine focal brain lesions by PrP^{Sc} accumulation and subsequent neuronal damage from only information about clinical manifestations.
- Another limitation of this study includes the relatively small number of plaque-type patients, which demonstrated no noteworthy results.

INTRODUCTION

Dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) is an acquired Creutzfeldt-Jakob disease (CJD) related to previous dura mater graft transplantation.[1 2] Details of abnormal prion protein (PrP^{Sc}) propagation in the human central nervous system (CNS) are not fully understood.[3] Previous studies of dCJD cases disclosing the relationship between initial manifestation and the site of grafting proposed that PrP^{Sc} may propagate directly from the contaminated dura mater graft to the adjacent brain regions and spread from the initially infected regions to